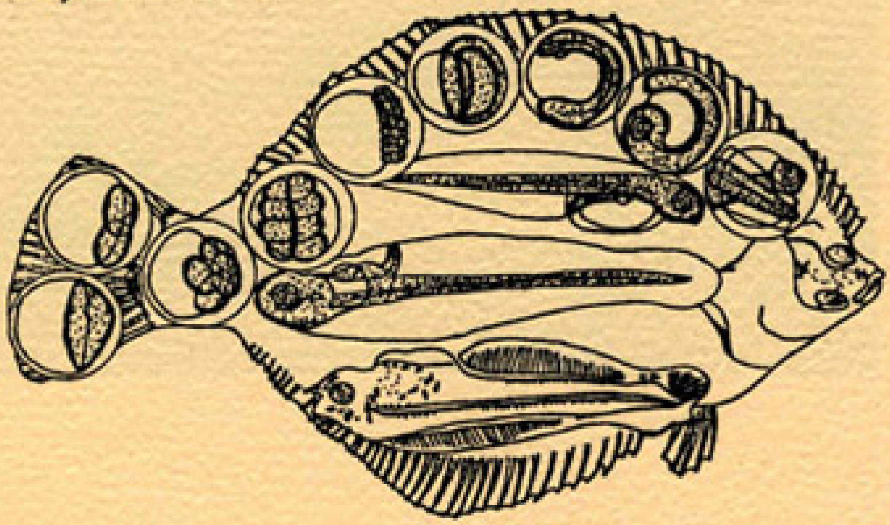
Winter Flounder Biology Workshop

December 3-4, 1991
Mystic, Connecticut



Program and Abstracts

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Winter Flounder Biology Workshop
December 3-4, 1991, Mystic, Connecticut

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Third in a series of Flatfish Biology Conferences

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Winter Flounder Biology Workshop

December 3-4, 1991, Ramada Inn, Mystic, Connecticut

Oral Presentations

Tuesday, December 3rd

8:00 a.m.	**Registration/Coffee-Ramada Inn**
9:00 a.m.	Welcome and Introduction **Robert A. Murchelano, Chief, Environmental Processes Division** National Marine Fisheries Service Woods Hole, MA **Anthony Calabrese, Conference Chair** National Marine Fisheries Service Milford, CT

Session I

Anne Studholme, Chair
National Marine Fisheries Service
Sandy Hook, NJ

9:30 a.m.	Habitat Issues in the Management of Inshore Stocks of Winter Flounder **P. Howell** *Connecticut Department of Environmental Protection, Waterford, CT*
10:00 a.m.	Vertical Distribution of Yolk-sac Winter Flounder Larvae in the Niantic River, CT **J. D. Miller** *Northeast Utilities Environmental Laboratory, Waterford, CT*
10:30 a.m.	**Coffee Break**

Session II

Allan Beck, Chair
Environmental Advantage Group
Prudence Island, RI

10:45 a.m.	Effects of the Bloom of the Diatom *Stephanopyxis turris* in Narragansett Bay in 1988 on Winter Flounder Larvae **G. Klein-MacPhee and E. Hjorleifsson** *University of Rhode Island, Narragansett, RI*
11:15 a.m.	Post-metamorphic Success in Winter Flounder: Identifying the Selective Agents **R. C. Chambers, D. F. Bertram, and W. C. Leggett** *McGill University, Montreal, Quebec, Canada*

11:45 a.m. Predation on Juvenile Winter Flounder: Effects of Prey Size and Predator Density
D. A. Witting and K. W. Able
Rutgers University Marine Field Station, Tuckerton, NJ

12:15 p.m. **Hosted Lunch**

Session III

Jay Burnett, Chair
National Marine Fisheries Service
Woods Hole, MA

1:30 p.m. Habitat Relationships in YOY Winter Flounder
L. A. Deegan[1] and S. E. Saucerman[2]
[1]Marine Biological Laboratory, Woods Hole, MA and [2]University of Massachusetts, Amherst, MA

2:00 p.m. Estimation of Winter Flounder Spawning Stock Abundance in the Niantic River
D. J. Danila
Northeast Utilities Environmental Laboratory, Waterford, CT

2:30 p.m. Growth and Maturation of Winter Flounder in Massachusetts Waters
D. Witherell[1] and J. Burnett[2]
[1]Massachusetts Division of Marine Fisheries, Sandwich, MA and [2]National Marine Fisheries Service, Woods Hole, MA

3:00 p.m. **Coffee Break and Poster and Exhibit Set-up**

Session IV

Don Danila
Northeast Utilities Environmental Laboratory
Waterford, CT

3:30 p.m. The Reproductive Cycle in Winter Flounder off Newfoundland
M. Burton
Memorial University of Newfoundland, St. John's, Newfoundland, Canada

4:00 p.m. Exploratory Stimulation of Some Environmental and Biotic Factors Affecting YOY Winter Flounder Growth and Survival
K. Rose[1], C. Chambers[2], Tyler[1], G. Klein-MacPhee[3], and D. Danila[4]
[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]McGill University, Montreal, Quebec, Canada, [3]University of Rhode Island, Narragansett, RI and [4]Northeast Utilities Environmental Laboratory, Waterford, CT

4:30 p.m. Winter Flounder Trophodynamics in the Stressed New York Bight Apex
F. Steimle
National Marine Fisheries Service, Sandy Hook, NJ

5:00 p.m. **Poster and Exhibit Set-up**

5:30 p.m. **Hosted Mixer and Poster and Exhibit Session**

Wednesday, December 4th

8:00 a.m. **Registration/Coffee**

Session V

Arnold Howe, Chair
Massachusetts Division of Marine Fisheries
Sandwich, MA

8:30 a.m. Effects of Environmental Salinity and Thyroid Axis Manipulation on Survival and Growth in Juvenile Winter Flounder
T. A. Whitesel, K. W. Able, and M. L. Keefe
Rutgers University Marine Field Station, Tuckerton, NJ

9:00 a.m. Questions on Variability in MFO Measurements from Wild Winter Flounder Stocks in Nova Scotia Waters
J. H. Vandermeulen, D. Mossman and V. Vignier
Bedford Institute of Oceanography, Department of Fisheries and Oceans, Dartmouth, Nova Scotia, Canada

9:30 a.m. Effects of Organochlorine Pesticides on Active Organic Anion Secretion by Primary Cultures of Winter Flounder Proximal Tubules
M. A. Dawson[1, 2] and J. L. Renfro[2]
[1]National Marine Fisheries Service, Milford, CT and [2]University of Connecticut, Storrs, CT

10:00 a.m. **Coffee Break**

Session VI

Chris Powell, Chair
Rhode Island Division of Fish and Wildlife
Kingston, RI

10:30 a.m. Antibody Levels Against Bacterial Pathogens are Increased in Winter Flounder from Polluted Sites
R. A. Robohm and D. Kapareiko
National Marine Fisheries Service, Milford, CT

11:00 a.m. The Cytopathology of Fin Erosion Disease in Winter Flounder
J. E. Bodammer
URI/ NOAA Cooperative Marine Education and Research Program, Kingston, RI

11:30 a.m. Hydropic Vacuolation in the Liver of Winter Flounder
M. J. Moore and J. J. Stegeman
Woods Hole Oceanographic Institution, Woods Hole, MA

12:00 p.m. Risk of Disease in Winter Flounder of Northeast Estuaries
S. A. MacLean and C. Meise-Munns
National Marine Fisheries Service, Narragansett, RI

Poster and Exhibit Session
Tuesday, December 3, 5:30 p.m.

Posters

Cloning of Two P-glycoprotein Genes in Winter Flounder (*Pseudopleuronectes americanus*)
K. M. Chann[1], P. L. Davies[1], L. Veinot[2], and V. Ling[2]
[1]Queen's University, Kingston, Ontario, Canada, and [2]The Princess Margaret Hospital and University of Toronto, Toronto, Canada

Development of a Population Model for Anthropogenic Effects Upon Reproduction, Growth and Survival of Winter Flounder in Long Island Sound
Staff
National Marine Fisheries Service, Northeast Fisheries Science Center

Numerical Response of Winter Flounder to Herring Spawn in the Southern Gulf of St. Lawrence
R. Morin[1], R. Tallman[2] and D. K. Cairns[1]
[1]Gulf Fisheries Centre, Department of Fisheries and Oceans, Moncton, New Brunswick, Canada and [2]Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba, Canada

Exhibits

Ultrasonic tags
Image analysis
Beam trawls

Abstracts

Oral Presentations

Habitat Issues in the Management of Inshore Stocks of Winter Flounder

P. Howell

Department of Environmental Protection
Marine Fisheries Office
PO Box 248, Waterford, CT 06385

Habitat issues usually present an intractable problem for fisheries managers. Although all management plans recognize that productive habitat is needed, and all losses are undesirable, weighing the importance of losses due to poor environmental conditions against losses due to fishing is extremely difficult. Analysis of flounder life history shows that under present levels of fishing mortality, an increase or decrease of young-of-the-year mortality is roughly equivalent to the same relative change in fishing mortality in terms of its effect on lifetime egg production. For this species, conditions of high adult fishing mortality may make early life stage losses more critical.

Review of the winter flounder literature identified three anthropogenic mortality factors exerting long-term deleterious effects on winter flounder habitat quality: toxic contamination of nearshore waters, habitat loss and alteration, and power plant entrainment and impingement. Specific effects are quantified as much as possible, but further study incorporating these mortality factors into fishery yield models is needed.

Vertical Distribution of Yolk-sac Winter Flounder Larvae in the Niantic River, CT

J. D. Miller

Northeast Utilities Environmental Laboratory
Millstone Nuclear Power Station
PO Box 128, Waterford, CT 06385

Yolk-sac winter flounder larvae were sampled at discrete depths in the Niantic River using a specially designed pump sampler. The sampler design allowed the collection of larvae prior to their passage through the pump, which would destroy yolk-sac winter flounder larvae. The purpose of the study was to determine if yolk-sac larvae congregated at the sediment-water interface. This behavior would retard their flushing from the river.

Twelve sets of samples were collected at three stations during February and March of 1991. Each set of samples consisted of collections at the surface, mid-depth, near bottom (approximately 0.3 m above the bottom), and at the sediment-water interface. Generally, the highest larval densities were found at near-bottom (geometric mean=214 per 500 m^3) and at mid-depth (324 per 500 m^3), with the lowest densities at the sediment-water interface (95 per 500 m^3) and at the surface (23 per 500 m^3). Densities at the sediment-water interface were significantly (a< 0.05) lower than at near-bottom and mid-depth. It did not appear, therefore, that yolk-sac larvae utilized vertical positioning in the water column as a retention mechanism.

Effects of a Bloom of the Diatom *Stephanopyxis turris* in Narragansett Bay in 1988 on Winter Flounder Larvae

G. Klein-MacPhee and E. Hijorleifsson

University of Rhode Island
Graduate School of Oceanography
Narragansett, RI 02882

A phytoplankton bloom of the diatom Stephanopyxis turris occurred in April 1988 in Narragansett Bay, Rhode Island. This diatom is part of the natural plankton community of Narragansett Bay, but normally occurs in low numbers and rarely dominates the spring plankton bloom. Winter flounder larvae and zooplankton collected April 1 and 4 in the Providence River were relatively immobile and appeared anesthetized in the sorting trays compared to fish collected at Greenwich Bay and Wickford Harbor on the same dates. The bloom traveled down the bay in the succeeding week and occurred in the Marine Ecosystems Laboratory mesocosms where winter flounder experiments were in progress.

The bloom caused a depression in the numbers of winter flounder larvae collected in the Providence River and a decrease in the medium length of each stage on two sampling dates after peak bloom. Effects on feeding habits of winter flounder larvae in the field and in the MERL mesocosms are discussed.

Post-metamorphic Success in Winter Flounder: Identifying the Selective Agents

R. C. Chambers, D. F. Bertram, and W. C. Leggett

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The age, size, and date at which winter flounder metamorphose from the larval to juvenile stage are known to vary. Evaluating the consequences of this variation is the next significant challenge. The approach employed here uses data from laboratory rearings as estimates of expected post-metamorphic growth dynamics, and applies principles appropriate for the analysis of longitudinal data. Based on simulations, we demonstrate expected size distributions of young-of-the-year juvenile flounder from two scenarios of size-selective mortality: 1) size-specific mortality operating on recently metamorphosed juveniles, and 2) size-specific mortality operating via winterkill. We detail implications of these two scenarios and suggest field tests for discrimination between these mechanisms.

Predation on Juvenile Winter Flounder: Effects of Prey Size and Predator Density

D. A. Witting and K. W. Able

Rutgers University, Marine Field Station
Institute for Marine and Coastal Sciences
PO Box 278, Tuckerton, NJ 08087

Preliminary laboratory and field observations have suggested that the sand shrimp (*Crangon septemspinosa*) may be an important predator of juvenile winter flounder (*Pleuronectes americanus*). Two laboratory experiments were conducted to investigate this predator-prey relationship further. The first experiment tested the effect of winter flounder stage/size on rate of predation by similar sized shrimp (45-55 mm TL). Flounder stages ranged from pelagic larvae (eyes symmetrical, 5-8 mm SL), to 60 mm (SL) juveniles. Experiments were conducted in replicate cylinders (0.2 m^2 and 51 cm in depth). The highest predation was observed on winter flounder that were at pre-eye and post-eye migration stages, with lower levels of predation observed during eye migration and on larger juveniles. These experiments suggest a complex interaction between winter flounder size, stage, settlement behavior, and susceptibility to predation by sand shrimp.

The second experiment tested the effect of shrimp density on predation rate. Six predator densities (0-36 m^2) were tested in pools (1 m^2 and 16 cm in depth). Winter flounder size and density was held constant (11-13 mm and 5 m^2, respectively). When predator densities were lower than 9 m^{-2}, flounder mortality was low (0-20%); however, in treatments where shrimp densities were 9 m^{-2} or greater, flounder mortality increased to nearly 100%. These results demonstrate that predator density has a pronounced effect on survival of juvenile winter flounder, therefore, inter- and intra- habitat variation in predator density could play an important role in determining settlement and survival of juvenile winter flounder in the field. Together these laboratory experiments substantiate the significance of predator–prey interactions to survival of young-of-the-year winter flounder. Further field studies are underway to define the extent and role of these interactions in winter flounder population dynamics.

Habitat Relationships in YOY Winter Flounder

L. A. Deegan[1] and S. E. Saucerman[2]

[1]*The Ecosystems Center*
Marine Biological Laboratory
Woods Hole, MA 02543

[2]*University of Massachusetts*
Department of Forestry and Wildlife Management
Amherst, MA 01003

Lateral and cross-channel exchange of young-of-the-year winter flounder were examined over a 40-day period in June and July, 1988, on Eel Pond, Massachusetts. Flounder were injected subcutaneously with two colors of acrylic paint and released at two locations on opposite sides of a channel. Recapture occurred one and three weeks after the last release date. Of the 275 recaptured fish, 98% were within 100 m of the release site, two (0.73%) were found on opposite sides of the channel, and three (1.09%) were found more than 200 m from the release site. Both release sites were near eelgrass beds, and the largest number of fish captured for marking were captured in the eelgrass areas. The highest percentage of recaptures were at the eelgrass beds where they had been initially captured. These results indicate that lateral and cross-channel movements of Y-O-Y winter flounder are limited in the summer months, and suggests that eelgrass plays an important role as nursery habitat for this species.

Twelve sites were studied in 1987 and 1988 at Waquoit Bay, Massachusetts, to evaluate age 0 winter flounder nursery habitats. The sites were classified as sand, silt or mud habitats, and as high, medium or low sediment organic content habitats. Significant differences were found in abundance, growth and condition of juvenile winter flounder at different sediment textures and levels of organic content. Abundance of juvenile winter flounder was highest in mud habitats with high organic content, and growth was lowest in the same habitat type. Condition of age 0 winter flounder was highest in high organic content habitats. These results illustrate the relative importance of different habitat types to juvenile winter flounder, and lay the groundwork for experimental research in this area.

Estimation of Winter Flounder Spawning Stock Abundance in the Niantic River

D. J. Danila

Northeast Utilities Environmental Laboratory
Millstone Nuclear Power Station
PO Box 128, Waterford, CT 06385

Abundance surveys of winter flounder spawning in the Niantic River, Connecticut have been made since 1976. These data have been used in the assessment of the operational impact of the Millstone Nuclear Power Station on this stock of winter flounder. Fish were obtained using otter trawls for mark and recapture experiments in which specimens larger than 15 or 20 cm were marked with a liquid nitrogen freeze brand for recapture in subsequent years. Absolute abundance estimates were made using the Jolly model for open populations. The trawl catch data were also used to calculate annual estimates of catch-per-unit-effort (CPUE). The Jolly absolute abundance estimates are considered reliable only since 1984 because of changes in sampling techniques and data collection methods, and both the Jolly estimate and CPUE are biased to some degree.

Population size ranged from about 36 to 80 thousand fish during 1984-90. A relative abundance index for female spawners, termed the annual standardized catch, was calculated from the trawl catches using abundance, gender, length-frequency, and maturity-at-size information. These annual indices made up a relatively consistent fraction (geometric mean of 3.5%; range of 2.7-4.5%) of the Jolly absolute population estimates. The relationship enabled the calculation of absolute female spawning stock size back to 1977 by extrapolation from the annual standardized catches, numbers of female spawners have ranged from about 19 to 79 thousand each year. The spawning population peaked in the early 1980s, as several large year-classes produced in the later 1970s were recruited to the spawning stock.

Growth and Maturation of Winter Flounder in Massachusetts Waters

D. Witherell[1] and J. Burnett[2]

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[2]*National Marine Fisheries Service*
Northeast Fisheries Science Center
Woods Hole Laboratory
Woods Hole, MA 02543

Growth and maturation of winter flounder were analyzed from data collected during the 1983-91 Massachusetts Division of Marine Fisheries spring inshore bottom trawl surveys. Data were analyzed separately for both Massachusetts stock units (north and south of Cape Cod). A von Bertalanffy growth model was fitted to length at age data for males and females of each stock unit. Analysis showed that winter flounder from the southern stock grew faster than those from the northern stock, and females grew faster than males. Mean lengths at age and calculated growth patterns for each sex were similar to those estimated from 1964-68 coastal tag return information. Annual variability in mean lengths at age was minimal, and was primarily due to changes in exploitation patterns resulting from regulations rather than changes in abundance or recruitment. Observed maturity at length data were well fitted by logistic models. Lengths at 50% maturity calculated from the curves were 28.5 and 29.3 cm for females, and 29.4 and 27.9 cm for males from the southern and northern stocks, respectively. Ages at 50% maturity were 3.1 years and 3.4 years for females from the southern and northern stocks, respectively, and 3.3 years for males of both stocks. These results show that Massachusetts winter flounder grow at faster rates and mature at larger sizes than has been reported for all other coastal populations.

The Reproductive Cycle in Winter Flounder off Newfoundland

M. Burton

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Newfoundland winter flounder spawn shortly after the end of their winter fast. If gametogenesis then occurs for the next spawning season, it is initiated for females during the summer feeding season with vitellogeneisis established, for Avalon Peninsular fish, by the end of August in recent years. Males undergo rapid spermatogenesis towards the end of the feeding season and hold activable sperm during the winter fast. Wild and laboratory held winter flounder may omit a spawning cycle in response to a poor feeding season.

Exploratory Simulation of Some Environmental and Biotic Factors Affecting YOY Winter Flounder Growth and Survival*

K. Rose[1], C. Chambers[2], J. Tyler[1], G. Klein-MacPhee[3], and D. Danila[4]

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YOY winter flounder population dynamics were modeled by individual-based Monte Carlo simulation. The model begins with the spawning of individual females, and follows each female-cohort of eggs through the yolk-sac stage until initiation of first feeding. At first feeding, individual larvae are sampled, and the growth and survival of individual feeding larvae are followed day-by-day through metamorphosis until the end of the year. Growth is represented with a bioenergetics model, with prey consumption resulting from stochastic prey encounters and captures. Survival of individual larvae is dependent on weight ("starvation") and length ("predation"). Niantic River and Narragansett Bay information was used to specify the environmental variables of temperature and prey types and densities. Model predictions under "typical" conditions were corroborated against Niantic River densities and length distributions. Exploratory simulations were performed comparing the effects on end-of-the-year survivors of: (1) number, sizes, and timing of spawning females, (2) interannual differences in daily water temperature, and (3) intensity of mortality during the larval period. Preliminary results suggest that varying combinations of these factors simultaneously can have interactive or synergistic effects on winter flounder dynamics during their first year of life.

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Winter Flounder Trophodynamics in the Stressed New York Bight Apex

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Highlands, NJ 07732

There is limited information of effects of sewage sludge disposal in coastal waters on trophodynamics of fishery resources. Possible effects include diet or feeding alterations in response to substances in the sludge or bottom water quality conditions, *e.g.,* hypoxia, promoted by sludge deposition and microbial degradation. During a three-year sewage sludge disposal cessation study in the New York Bight apex, stomach contents of 4,189 winter flounder were examined from bimonthly collections at three stations in the apex.

Results indicate a mixed response to cessation, with about a 100% increase in percent empty stomachs (a possible indicator of feeding inhibition) at the site closest to the former disposal areas and a 30 to 40% decrease at two stations several miles away. The composition of the diet showed no major changes in dominant prey (cerianthiid anemones, nemertians, and several larger polychaetes) during the study, although the percent frequency of occurrence (%FO) of *Capitella* sp. (a stressed environment indicator) decreased from means of 3-19% during disposal to <1% at all stations after cessation. The %FO of some amphipod species (possible indicators of relatively unstressed environments) increased from means of 0.7-6.7% during disposal to 4.9-36.8% after cessation.

These responses closely correspond generally to changes in the population abundances of these prey in the benthic community monitored during the study. All benthic community changes were not, however, reflected in the diet composition, *e.g.,* mollusc population variability. Preliminary data on chemical contaminant body burdens of major prey from the former disposal area and nearby areas suggest this factor is important and probably contributes to the chemical contamination of winter flounder tissue reported for the area.

Effects of Environmental Salinity and Thyroid Axis Manipulation on Survival and Growth in Juvenile Winter Flounder

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This study examined the influence of environmental salinity and the thyroid axis on the survival and growth of juvenile winter flounder (*Pleuronectes americanus*). Metamorphic larval flounder were obtained on 28 March 1991, from the National Marine Fisheries Service (Milford, Connecticut) and reared at Rutgers Marine Field Station (Tuckerton, New Jersey).

The range of salinities which flounder could tolerate was examined in an initial set of experiments in May. When fish were directly transferred from ambient seawater (SW) (29-31 ppt) to 0, 1, 3, 4, 6, 7, 14, 21, 28 and 35 ppt SW, only fish in the 0 and 1-ppt treatment died within120 h, whereas fish in 6-35 ppt SW all survived for 120 h. Fish that were gradually acclimated to lower salinities began to die in 3.5 ppt SW and no fish survived at a salinity less than 1.75 ppt.

A second set of experiments examined the effect of manipulating the thyroid axis on the survival and growth of juvenile flounder over the range of salinity they could tolerate. Fish were transferred directly from 29 ppt SW to 2, 7, 21, and 35 ppt SW, as well as to a saline environment that fluctuated between 15 and 30 ppt (15/30) every 48 h; treated with 0.1 ppm thyroxine (T_4), 30 ppm thiouracil (TU) or as a sham (C); and held for either 120 h or 16 d. No effect of thyroid manipulation was seen on the survival in any salinity within 120 h. However after 16 d, treatment with TU increased the mortality observed in 7 and 35 ppt SW whereas mortality was minimal in the other treatments. After 16 d treatment with TU also enhanced the absolute growth of fish in 21 and 15/30 ppt SW whereas absolute growth was not affected by the other treatments.

These data confirm that juvenile winter flounder are not euryhaline but suggest they can survive and grow equally well in salinities ranging from 6 or 7 to 35 ppt. Furthermore, the effect of the thyroid axis on growth and survival appears to be modified in a complex manner by environmental salinity.

Questions on Variability in MFO Measurements from Wild Winter Flounder Stocks in Nova Scotia Waters

J. H. Vandermeulen, D. Mossman, and V. Vignier

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In the course of 5 years of MFO and related measurements (total cyt. P450) in winter flounder from several locations on the Scotian Shelf, we have encountered very wide swings in variability, including from year to year. Factors influencing this variability appear to be season, sex, state of maturity, and age. We have been able to maintain low enough levels of variability in our laboratory induction work to use both EROD and AHH (BaPH) as measures of environmental condition. Our observations raise questions about the use of MFO measurements in wild migratory fish stocks with unknown feeding and exposure histories.

Effects of Organochlorine Pesticides on Active Organic Anion Secretion by Primary Cultures of Winter Flounder Proximal Tubules*

M. A. Dawson[1, 2] and J. L. Renfro[2]

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Northeast Fisheries Science Center
Milford Laboratory, Milford, CT 06460

[2]*University of Connecticut*
Marine/Freshwater Biomedical Sciences Center
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Storrs, CT 06268

A number of potentially toxic compounds, both endogenous and xenobiotic, are secreted into the urine as organic anions by the renal proximal tubule. Such secretion reduces the total body burden of these toxic substances; accumulation of certain organic anions in kidney cells and urine in the course of this secretion may subject the kidney to damage. We tested the potential of this system to transport four closely related organochlorine pesticides, using inhibition of p-aminohippuric acid (PAH) secretion as a criterion. The pesticides tested were 2, 4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4-dichlorophenoxy) propionic acid (dichloroprop), 2-methyl, 4-chlorophenoxyacetic acid (MCPA), and 2-(2-methyl, 4-chlorophenoxy) propionic acid (mecoprop). PAH flux was measured at a PAH concentration of 10 µM in the presence of 100 µM of each pesticide. Three of the four inhibited PAH flux at the latter concentration and were further tested at pesticide concentrations ranging from 0.1 µM to 1.0 mM. MCPA has no significant effect at 100 µM and was not tested further.

The use of cultures allowed us to mount the tissues in Ussing chambers and to measure unidirectional fluxes in the absence of electrochemical gradients. We monitored tissue concentration throughout each experiment by measurement of transepithelial potential difference (PD) and resistance (R). At the end of each experiment, as a further indication of tissue condition, we measured sodium-dependent glucose transport, as indicated by phloridzin-sensitive short circuit current (PSC).

2, 4-D at concentrations of 0.1 and 1.0 mM inhibited PAH secretion by 20% and 80%, respectively. Lower concentrations had no significant effect on secretion. No 2, 4-D concentration tested significantly affected reabsorption, PD, R, or PSC.

Dichlorprop inhibited PAH secretion at concentrations of 10 µM and above. Inhibition ranged from 19% at 10.0 µM to 77% at 1.0 µM. No dichloroprop concentration tested changed reabsorptive flux, PD, R, or PSC.

Mecoprop inhibited PAH secretion by 18% at 0.1 mM and by 80% at 1.0 mM with no effect on reabsorption, PD, R, or PSC. At mecoprop concentrations of 0.1 and 1.0 µM, PAH flux increased steadily through the experiment, reaching 139 and 132% of control, respectively, at 120 minutes. The simplest explanation, as yet untested, is that intracellular mecoprop accumulates and eventually increases PAH flux by anion exchange at the cytosolic face of either the peritubular or the luminal membrane. This biomonitoring system shows that these pesticides interact with anion secretion in the proximal tubule and may, through competition or stimulation, affect the dwell time of other toxins in the body.

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Antibody Levels Against Bacterial Pathogens are Increased in Winter Flounder from Polluted Sites

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The sera of 832 winter flounder, *Pleuronectes americanus*, from Long Island Sound and Boston Harbor were tested for antibodies against a panel of eight bacterial pathogens. Antibody titers were determined by agglutination reactions against formalin-fixed bacteria. The presence of antibodies (rather than non-specific agglutinins) was confirmed by treating representative sera with 2-mercaptoethanol, an antibody-disrupting reagent. To avoid a temperature effect that could bias comparisons of antibody levels between the 10 sites of fish capture, serum titers were divided into 2 groups: [1] those from fish captured at temperatures above 5ºC and [2] those from fish captured at 5ºC or less. Site comparisons for both temperatures indicated that total antibody levels are generally proportional to the estimated degree of pollution at the site. For example, the progression of calculated antibody indices for sites of fish capture when the temperature was 5ºC or less was: Shoreham < Niantic < Milford < Madison < Boston [DI] < Hempstead < Norwalk=Boston [LI]: the antibody indices for Boston [LI], Norwalk, and Hempstead were all significantly greater than the index for Shoreham. For temperatures above 5ºC the progression was: Niantic < Shoreham < Bridgeport < New Haven; the antibody indices for Boston [LI], Norwalk, and Hempstead were all significantly greater than the index for Shoreham. For temperatures above 5ºC the progression was: Niantic < Shoreham < Bridgeport < New Haven; the antibody indices for Niantic and Shoreham were significantly less than those for Bridgeport and New Haven. This work indicates that, in winter flounder, the presence of higher mean antibody levels (against selected bacteria) signifies residence in anthropogenically-degraded environments.

The Cytopathology of Fin Erosion Disease in Winter Flounder

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High resolution light microscopic and fine structural studies were conducted on the early stages of fin erosion disease in winter flounder collected from Raritan Bay, the New York Bight, and New Haven Harbor, three severely contaminated sites along the Atlantic coast.

Fin tissues routinely demonstrated the following three types of lesions 1] frank necrosis resulting in severe epithelial sloughing; 2] epidermal hyperplasia (nonpapillomatous) with and without acantholysis; and 3] nonhyperplastic epithelia featuring middle-cell layer acantholysis accompanied by excessive accumulation of intracellular glycogen.

The underlying dermis also exhibited necrosis, blood vessel occlusion, and hemorrhage. A consistent absence of either bacteria or viruses in the epidermis and underlying lamina propria supports an idiopathic diagnosis for this disease at present; however, the putative effects of contaminants will be discussed.

Hydropic Vacuolation in the Liver of Winter Flounder

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Our previous studies of the development of liver disease in winter flounder, *Pleuronectes americanus*, from Boston Harbor revealed hydropically vacuolated cells to first appear in the biliary productular epithelia, which are found in the center of hepatic tubules. In larger and older fish, vacuolated cells were found throughout hepatic tubules and in grossly visible nodules. Vacuolated cells were intimately associated with a diverse array of cholangiocellular neoplasms, and the less common hepatocellular tumors. In this study, the ultrastructure and DNA synthetic activity of vacuolated cells was examined to investigate their potential role in the progression to neoplasia.

Ultrastructural studies showed that the development of vacuolation involved the accumulation of electronlucent material within the cisternae of the endoplasmic recticulum, in the perinuclear space, and in mitochondria.

The potential for these cells to proliferate was examined by developing an immunohistochemical assay to detect nuclei actively synthesizing DNA in S-phase. Flounder were pulse labeled with a nucleotide analog, bromodeoxyuridine (BrdU). Nuclear incorporation of BrdU was detected immunohistochemically. Vacuolated, and most particularly neoplastic nuclei, were found to have elevated numbers of nuclei that incorporated BrdU. It was concluded that this was evidence for replicative DNA synthesis, increased cell cycling, and possibly cell proliferation. The utility of the BrdU incorporation assay is currently being developed as an assay that will examine the linkage between epigenetic carcinogens and detrimental biological effects.

Risk of Disease in Winter Flounder of Northeast Estuaries

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Data on histopathological conditions of winter flounder collected from 11 Northeast estuaries (from Great Bay, NJ to Casco Bay, ME) during the National Status and Trends program were analyzed for years 1984 through 1986. Lesions in the gill, kidney and liver of 809 winter flounder were categorized by type (e.g., inflammatory, degenerative, proliferative) according to NS&T standards. Using the prevalence of lesions, the odds ratio was calculated as a measure of potential risk of developing some types of lesions when factored against estuarine collection site (an estimate of contaminant exposure) and size (an estimate of age). Contaminant exposure increased risk of some lesions, as did age alone. Some lesions showed no risk associated with these two factors.

Abstracts

Poster Presentations

Cloning of Two P-glycoprotein Genes in Winter Flounder (*Pleuronectes americanus*)

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P-glycoprotein (P-gp) is a membrane-spanning protein, which is responsible for the energy-dependent efflux of drugs, called multiple-drug resistance (Mdr), observed in mammalian cell lines, tumors or cancer cells. Recent studies have shown that both P-gp and cytochrome P-450 genes were inducible in rat liver following administration of xenobiotics. We have isolated P-gp gene specific probes from the winter flounder to test the hypothesis that P-gp can be induced by xenobiotics as part of the detoxification process in fish.

Southern blot analysis using a conserved 3'-terminal region of hamster P-gp CDNA (pEXI) as a probe revealed that there are two P-gp genes in right-eyed flounders. We have isolated two sets of clones from a winter flounder genomic library that correspond to the 3'-ends of the two flounder P-gp genes. Sequence analysis has been focused on two key areas: the 3'-ATP binding site and the pEXI region, both of which are homologous with their mammalian counterparts. These cloned sequences are the first set of P-gp genes reported in fish and will be useful for delineating the expression of P-gp genes in teleosts.

Development of a Population Model for Anthropogenic Effects upon Reproduction, Growth, and Survival of Winter Flounder in Long Island Sound

Staff

National Marine Fisheries Service
Northeast Fisheries Science Center

Environmental quality has been theorized to affect the stability of winter flounder populations in highly urbanized coastal areas. We propose to test this hypothesis in Long Island Sound using flounder from environmentally contaminated New Haven Harbor and from a “clean” area off Milford, Connecticut. Recent research has demonstrated abnormal biological function in the flounder, including reproductive impairment, at New Haven. We will strengthen existing evidence by expanding current field and laboratory research, and by simultaneously developing a conceptual winter flounder life history model to evaluate the hypothesis. The use of a life history model will help to organize the available information on anthropogenic effects upon winter flounder. The model will aid in interpreting data, and will generate logical predictions on anthropogenic effects at the population level- a necessary prediction that has been elusive.

The proposed study will be a collaborative effort between two laboratories in the NMFS Northeast Fisheries Science Center. The Milford Laboratory will conduct field and laboratory studies designed to provide data on reproduction, growth and survival of winter flounder, especially as these parameters relate to toxic contamination and environmental degradation. The Woods Hole Laboratory will conduct age and growth analyses and will develop the life history/population model.

Numerical Response of Winter Flounder to Herring Spawn in the Southern Gulf of St. Lawrence

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To test the hypothesis that a strong predator-prey linkage exists between winter flounder and herring spawn, we attempted to fit the Holling numerical response model to flounder predation on herring spawn deposited at Fisherman's Bank. P.E.I. Surveys using underwater video cameras and scuba divers demonstrated that we could predict both where and when herring would spawn. Interannual variation was limited to position of the spawning beds on the bank and fluctuations of a week or two in the start and end of spawning. Camera surveys showed that flounder were closely associated with deposited spawn. Examination of stomach contents revealed that flounder consumed large quantities of eggs. However, the density of flounder did not increase as a smooth function of the increasing density of herring eggs. Area trawl surveys revealed substantial numbers of potential flounder predators. We suggest that *in situ* study results do not fit a standard aggregative response pattern because flounder must trade off between increased vulnerability to predators when positioned on a patch of eggs and the pay-off from eating eggs. We propose that these results show that winter flounder are a major predator of herring spawn, but are constrained from achieving a maximal rate of harvest by the presence of predators. We also propose that herring may be concentrating reproduction in time and space to swamp potential predators. The successful evolution of mass spawning must have been accelerated because large spawning beds are risky places to operate as an egg predator.